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Staphylococcus aureus Isolates Carrying Panton-Valentine Leucocidin Genes in England and Wales: Frequency, Characterization, and Association with Clinical Disease

A. Holmes,* M. Ganner, S. McGuane, T. L. Pitt, B. D. Cookson, and A. M. Kearns

Laboratory of HealthCare Associated Infection, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London, NW9 5HT, United Kingdom

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Staphylococcus aureus **isolates carrying the genes that encode for Panton-Valentine leucocidin (PVL), a highly potent toxin, have been responsible for recent outbreaks of severe invasive disease in previously healthy children and adults in the United States of America and Europe. To determine the frequency of PVL-positive isolates sent to the Staphylococcus Reference Unit (United Kingdom) for epidemiological purposes, we tested 515 isolates of *S. aureus*, and 8 (1.6%) were positive for the PVL locus. A further 470 isolates were selected to explore the association of PVL-positive *S. aureus* with clinical disease. Of these, 23 (4.9%) were PVL positive and most were associated with skin and soft tissue infections (especially abscesses). The PVL genes were also detected in isolates responsible for community-acquired pneumonia, burn infections, bacteremia, and scalded skin syndrome. Genotyping by pulsed-field gel electrophoresis and multilocus sequence typing revealed that the PVL-positive isolates were from diverse genetic backgrounds, although one prevalent clone of 12 geographically dispersed methicillin-resistant *S. aureus* (MRSA) isolates was identified (ST80). All 12 isolates were staphylococcal cassette chromosome *mec* type IVc, had an *agr*3 allele, and shared a common toxin gene profile (*sea-see*, *seg-sej*, *eta*, *etb*, and *tst* negative but *etd* positive). ST80 strains with similar genetic characteristics have been responsible for community-acquired infections in France and Switzerland. The remaining PVL-positive isolates were mostly methicillin-sensitive *S. aureus* and belonged to 12 different sequence types, including ST22 and ST30, which are closely related to the most prevalent MRSA clones in United Kingdom hospitals, EMRSA-15 and EMRSA-16, respectively.**

Staphylococcus aureus is a very successful hospital and community-acquired pathogen. It causes a broad spectrum of disease, from mild skin infections to more serious invasive infections, including septicemia, pneumonia, endocarditis, and deep-seated abscesses. Pathogenicity is related to a number of virulence factors that allow it to adhere to surfaces, invade or avoid the immune system, and cause harmful toxic effects to the host. These factors include cell surface components (e.g., protein A, fibronectin-binding protein, collagen-binding protein, and clumping factor), and exoproteins (e.g., enterotoxins, exfoliatins, toxic shock syndrome toxin, and Panton-Valentine leucocidin [PVL]).

PVL is a pore-forming cytotoxin that targets human and rabbit mononuclear and polymorphonuclear cells (37). When injected intradermally into rabbits, it induces severe inflammatory lesions, leading to capillary dilation, chemotaxis, polymorphonuclear karyorrhexis, and skin necrosis (44). Studies have shown that its toxic effect results from the synergistic action of two separate exoproteins, namely, LukS-PV and LukF-PV. These proteins are encoded by two contiguous and cotranscribed genes (*lukS-PV* and *lukF-PV*) (36), which are carried on temperate bacteriophages (20). Lysogenic conversion of PVL-negative strains of *S. aureus* leading to the production of the toxin has been demonstrated (29).

* Corresponding author. Mailing address: Laboratory of Healthcare-Associated Infection, Centre for Infections, 61 Colindale Avenue, London, NW9 5HT, United Kingdom. Phone: 44 208 200 4400. Fax: 44 208 200 7449. E-mail: anne.holmes@hpa.org.uk.

Recently, there has been much interest in PVL, due to its involvement in severe disease among children and young adults with no known exposure to healthcare establishments. In the United States of America, outbreaks of severe skin infections have occurred in homosexual men, prison inmates, and schoolchildren (6). Similarly, PVL-related skin infections have been reported in the gay community in The Netherlands (43), in schoolchildren in Switzerland (3), and among healthcare staff in Scotland (40). Most worrying, however, is the increasing number of reports of PVL-positive strains associated with severe necrotizing community-acquired pneumonia. An association between *S. aureus* strains carrying the genes for PVL and community-acquired pneumonia was first noted by Lina et al. (27). They developed a PCR assay to detect the PVL genes and found a significant association between the presence of the locus and severe necrotic community-acquired pneumonia (8% of cases compared to no cases associated with hospital-acquired pneumonia). They also showed that the PVL genes were highly associated with primary cutaneous infections, especially furunculosis, confirming earlier findings by other workers who used double immunodiffusion to detect the toxin (7, 8, 35). More recently, cases of community-acquired pneumonia due to PVL-positive *S. aureus* have been reported in France (4, 14, 25), Sweden (32), The Netherlands (42), and the United Kingdom (23). In addition, the PVL genes have been identified as a stable marker of community-acquired methicillin-resistant *S. aureus* (MRSA) strains worldwide (41).

In 1995, Prevost et al. (35) reported that PVL was produced by 2% of *S. aureus* isolates in a general hospital in France.

TABLE 1. Association of PVL-positive isolates with types of staphylococcal infection

Type of infection	No. of strains	No. (%) of PVL-positive	Risk ratio (95% CI)[a]	*P* value
Skin and soft tissue infections	63	15 (24)	12.1 (5.4, 27.4)	<0.00005
Cellulitis	30	3 (10)	2.2 (0.7, 7.0)	0.17
Abscess	16	7 (44)	12.4 (6.0, 25.9)	<0.00005
Ulcer	6	0 (0)	0	1.00
Skin lesions/boils	11	5 (45)	11.6 (5.3, 25.5)	0.0001
Impetigo/blisters/eczema	50	0 (0)	0	0.16
Scalded skin syndrome	61	1 (2)	0.3 (0.04, 2.2)	0.34
Pneumonia	37	4 (11)	2.5 (0.4, 6.9)	0.10
Wounds[b]/burn	50	2 (4)	0.8 (0.2, 3.3)	1.00
Septicemia/bacteremia	65	1 (2)	0.3 (0.04, 2.1)	0.23
Endocarditis	41	0 (0)	0	0.25
Toxic shock syndrome	51	0 (0)	0	0.16
Food poisoning	15	0 (0)	0	1.00
Other invasive infections[c]	37	0 (0)	0	0.24
Total	470	23		

[a] Risk ratio is the ratio of the risk of being PVL positive in the presence of a particular type of infection to the absence of that type of infection. CI, confidence interval.
[b] Mostly postsurgical wounds.
[c] Including osteomyelitis and arthritis.

More recently, the PVL genes have been detected in 10% of MRSA isolates received by the Dutch Institute of Public Health and the Environment (43). The purpose of this study was threefold. Firstly, it was to determine the frequency of PVL genes among *S. aureus* isolates sent to the Staphylococcus Reference Unit from laboratories throughout England and Wales for epidemiological purposes (including surveillance, suspected outbreaks, unusual resistance, and suspected toxin-mediated disease). Secondly, it was to investigate the association of PVL genes with various types of staphylococcal disease. Thirdly, it was to determine the strain characteristics and genetic relatedness of the PVL-positive isolates.

MATERIALS AND METHODS

Bacterial isolates. Two different collections of isolates were used in this study. The first collection of 515 isolates was used to determine the frequency of PVL genes among *S. aureus* sent to the Staphylococcus Reference Unit for epidemiological purposes. The isolates were collected by selecting every tenth isolate from those received in 2002 until a reasonable sample size was obtained. Every tenth isolate was selected, to avoid undue bias through sampling isolates from the same outbreak, and a sample size of at least 500 was required to enable a reasonably precise estimate of PVL prevalence (e.g., if the prevalence was 2%, then the 95% confidence interval is 1% to 3.6%).

A second collection of 470 isolates was used to determine the distribution of PVL genes in different types of staphylococcal infections. The isolates were from 2002 through 2003 and different from those selected for the frequency study, except for 19 isolates, as many isolates received in the laboratory are from surveillance samples, for which either no clinical details or inadequate clinical details are provided. The 19 isolates common to the two studies were a consequence of different laboratory personnel selecting the samples independently. The 470 isolates were categorized into 10 different types of staphylococcal infection, based on the clinical details provided (Table 1). Approximately 50 isolates for each type of infection were required to enable any large differences in PVL prevalence to be detected (80% power at a 5% significance level to detect a difference between a PVL prevalence of 18% in the presence of a particular type of infection and 5% in the absence of that infection). Cases of skin infections were grouped according to whether they had been previously associated with PVL genes (e.g., abscesses or cellulitis) and those that had not (e.g., impetigo).

All isolates gave a characteristic appearance on CHROMagar STAPH AUREUS medium (Oxoid, Basingstoke, United Kingdom) (12).

DNA extraction. DNA was isolated by using either the DNAeasy tissue kit (QIAGEN, Crawley, United Kingdom) as recommended by the manufacturer, with the modification that 30 μg/ml of lysostaphin was added to the lysis buffer, or by using Chelex resin (Bio-Rad Laboratories, Hemel Hempstead, Hertfordshire, United Kingdom). For the latter, one loopful of growth from nutrient agar plates incubated overnight at 37°C was resuspended in 100 μl of 5% Chelex resin (Bio-Rad). The suspension was boiled for 10 min and centrifuged to remove cell debris. The cell lysate was used as the template for PCR.

PCR. All isolates were tested for the presence of PVL genes and the 16S rRNA gene as an amplification control by duplex PCR using previously described primers (27, 28). DNA amplification was performed on an Eppendorf MasterCycler in a final volume of 50 μl containing 1× PCR buffer (Invitrogen, Paisley, United Kingdom), 3 mM $MgCl_2$, 200 μM of each dNTP, 0.3 μM of primers *luk-PV-1* and *luk-PV-2*, 0.02 μM of 16S rRNA primers, 1 U of *Taq* DNA polymerase (Invitrogen), and 2 μl neat cell lysate or 1:10 diluted QIAGEN-extracted DNA. Samples were denatured for 5 min at 95°C, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min, with the final extension at 72°C for 5 min. PCR products were analyzed by electrophoresis through a 2% agarose gel (Bio-Rad).

Isolates that carried the PVL genes were subsequently analyzed for a variety of other staphylococcal genes. Four separate multiplex PCRs were used to detect the following genes: staphylococcal enterotoxin genes *sea*, *seb*, *sec*, *sed*, and *see* (reaction 1) (2); *seg*, *seh*, *sei*, and *sej* (reaction 2) (28); toxic shock syndrome toxin gene (*tst*) and exfoliatin toxin genes *eta* and *etb* (reaction 3) (2); and *etd* and *lukS-PV lukF-PV* (reaction 4) (27, 45). Each multiplex reaction contained the primers for the 16S rRNA gene as an internal control (28). The PCR conditions were as described above, except the primer concentrations varied between 0.05 μM and 0.3 μM. The accessory gene regulator (*agr*) allele group (1 through 4) and staphylococcal cassette chromosome (SCC*mec*) element (I through IV) were determined as previously described (31, 34). Isolates that carried the SCC*mec* type IV element were further tested with primers specific for subtypes IVa through IVd (15, 30) and type 2 cassette recombinase genes (*ccrA/B*) (16).

S. aureus strains NCTC 13300 (*lukS-PV lukF-PV*), NCTC 10652 (*sea agr*1), NCTC 10654 (*seb*), NCTC 10655 (*sec*), NCTC 10656 (*sed agr*2), ATCC 27664 (*see*), NCTC 11963 (*tst agr*3), Ty114 (*etd*), EMRSA-1 (SCC*mec* III), EMRSA-2 (SCC*mec* IV), EMRSA-3 (SCC*mec* I), EMRSA-16 (SCC*mec* II), MW2 (SCC*mec* IVa), and 8/6-3P (SCC*mec* IVb) and clinical isolates (*eta etb agr*4) were used as positive controls for PCR. SCC*mec* types I through IV in United Kingdom EMRSA isolates had been previously characterized (11). Ty114 and 8/6-3P were kindly provided by Motoyuki Sugai (Hiroshima University, Japan) and Teruyo Ito (Juntendo University, Japan), respectively.

Antimicrobial susceptibility testing. Susceptibility to a range of antimicrobial agents was determined by the agar dilution method using diagnostic sensitivity test supplemented with 2% lysed blood and incubated at 37°C, except for plates containing methicillin and oxacillin, which were incubated at 30°C for 48 h.

Phage typing. Phage typing was performed with the 23 phages of the Basic International Set (33) at 100× routine test dilution.

PFGE. Pulsed-field gel electrophoresis (PFGE) profiles were obtained by macrorestriction of chromosomal DNA with SmaI and a run time of 30 h with pulse switch times of 1 to 80 s (22). Banding patterns were digitalized and analyzed using BioNumerics (Applied Maths, Ghent, Belgium). Profiles that were >80% similar were considered closely related.

MLST. Multilocus sequence typing (MLST) was performed as described by Enright et al. (10). PCR products were purified using the QIAGEN PCR purification kit and both strands were sequenced on the CEQ 8000 genetic analysis system (Beckman Coulter, High Wycombe, United Kingdom). BioNumerics (Applied Maths) was used to align and analyze the sequences. Allelic profiles and sequence type (ST) designations were assigned by comparison with previously characterized strains using the MLST database via the internet (http://www.mlst.net).

Statistical analyses. Frequencies of PVL genes were calculated with 95% confidence intervals. Fisher's exact tests were performed to determine associations of the presence of PVL genes with types of staphylococcal infections. Risk ratios with 95% confidence intervals were calculated by comparing the risks of PVL positivity in those with and without each type of infection studied.

RESULTS

Frequency of PVL genes among referred isolates. A total of 515 isolates of *S. aureus* selected from those referred for epidemiological purposes in 2002 was tested for the presence of

the PVL genes by PCR, and 8 (1.6%) were positive. The 95% confidence interval for positivity was 0.7% to 3.0%.

Distribution of PVL genes in staphylococcal disease. Of the 470 isolates of *S. aureus* from different types of staphylococcal infection, 23 (4.9%) were PVL positive and the majority (15 [65%]; risk ratio, 12.1; 95% confidence interval, 5.4 to 27.4; $P < 0.00005$) were associated with skin and soft tissue infections (abscesses [$n = 7$], cellulitis [$n = 3$], skin lesions [$n = 3$], and boils [$n = 2$]) (Table 1). The remaining isolates were from cases of pneumonia ($n = 4$), burn infections ($n = 2$), bacteremia ($n = 1$), and scalded skin syndrome (SSS; $n = 1$). PVL genes were not detected in isolates associated with deep-seated infections, such as endocarditis, osteomyelitis, or arthritis, nor in those causing impetigo, toxic shock syndrome, or food poisoning.

Characterization of *S. aureus* strains carrying PVL genes. A total of 30 isolates tested positive for the PVL genes; one of the PVL-positive isolates detected in the frequency study was among those chosen to determine the distribution of PVL genes in staphylococcal disease (no. 28) (Table 2). Clinical details were available for four of the seven other isolates detected in the frequency study. Two of the isolates were from patients with bacteremia, one was from a patient with empyema, and one was from a patient with skin lesions (Table 2).

Antimicrobial susceptibility patterns. The antimicrobial susceptibility patterns differed among the PVL-positive isolates. Fourteen (47%) were resistant to methicillin and oxacillin, which was confirmed by the presence of the *mecA* gene. One isolate appeared methicillin/oxacillin sensitive phenotypically but carried the *mecA* gene (no. 30) (Table 2). Several of the isolates were resistant to chloramphenicol (43%), tetracycline (40%), fusidic acid (33%), gentamicin (20%), and erythromycin (13%), while resistance to quinupristin-dalfopristin, ciprofloxacin, or mupirocin was rare (all 3%). All isolates were susceptible to vancomycin and teicoplanin. Two isolates were susceptible to all of the antibiotics tested.

Accessory gene detection. The most frequently encoded toxin genes among the PVL-positive *S. aureus* isolates were *seg* (50%), *sei* (50%), and *etd* (40%) (Table 2). However, *tst*, *sea*, *seb*, *sec*, and *seh* were encoded less frequently, and *eta*, *etb*, and *sej* were not present in any of the isolates. Two of the isolates did not carry any of the 13 toxin genes.

Typing of the *agr* locus, which controls the expression of many of the virulence factors of *S. aureus*, showed that the majority of the isolates (70%) had *agr*3, including the 12 *etd*-positive isolates. Previous studies have shown that an *agr*3 background is common among community-acquired MRSA isolates (9, 26, 41). Six isolates had *agr*1, including the three ST22 strains. The majority of the United Kingdom epidemic healthcare-associated MRSA isolates had *agr*1 (unpublished data). The three phage group II isolates had *agr*4, and only one isolate had *agr*2.

Typing of isolates carrying PVL genes. Twenty-five (83%) of the thirty strains were typeable by one or more of the phages at 100× routine test dilution (Table 2). Of these typeable strains, 8 belonged to group I, 3 to group II, 4 to group III, and 10 to more than one group (Table 2). SmaI macrorestriction analysis identified 14 different genotypes, A through N (Fig. 1). The 12 MRSA strains harboring the genes that encode for PVL and ETD clustered at 74%, and the majority belonged to phage group I, suggesting that they were related. This was confirmed by MLST and SCC*mec* typing, as they all belonged to the same sequence type (ST80) and carried the SCC*mec* IVc element. Among the other 18 strains, 12 different sequence types were identified, 9 of which included only a single isolate. The three phage group II methicillin-sensitive *S. aureus* strains belonged to ST121. Three methicillin-sensitive *S. aureus* strains belonged to ST22, which is closely related to EMRSA-15, the most common epidemic clone currently circulating in British hospitals. Similarly, three strains belonged to ST30, which is closely related to the second major British clone EMRSA-16 (ST36); ST30 differs from ST36 in only one of the seven loci. One of the ST30 strains was resistant to methicillin and carried the SCC*mec* IVc element. Three isolates were assigned new sequence types (ST359, ST360, and ST361). The ST359 strain was *mecA* positive but did not match the SCC*mec* types described by Oliveira and de Lencastre (31). Further analysis of this strain showed it did not carry the recombinase genes *ccrA* and *ccrB* described by Ito et al. (16), so we classified it as nontypeable.

Geographical distribution. The 30 PVL-positive *S. aureus* isolates were from 22 different healthcare centers throughout most regions of England and Wales. The 12 ST80 strains were from nine different centers, and there was no known epidemiological link between the ST80 strains from the same centers.

DISCUSSION

The Staphylococcal Reference Unit receives approximately 9,000 isolates of *S. aureus* each year from clinical laboratories throughout England and Wales for epidemiological typing and toxin gene testing. The results of this study showed that only a small proportion (1.6%) of a selected sample of these isolates harbors the genes that encode PVL, which is similar to reports from other countries. It has been suggested that the reason for this is that only a few *S. aureus* strains are susceptible to infection with PVL-converting phages. Narita et al. (29) showed that the temperate phage ϕSLT infected only 3% of clinical PVL-negative *S. aureus* strains, leading to PVL production. Different strains of *S. aureus* have been shown to harbor different PVL-carrying phages (20, 21, 29, 46). However, it is unknown whether these phages are capable of infecting PVL-negative strains, resulting in expression of the toxin.

When isolates were categorized according to type of staphylococcal infection, the PVL genes were strongly associated with skin and soft tissue infections, such as abscesses, skin lesions, and boils (furuncles). By contrast, no statistically significant association was observed with impetigo, blisters, or SSS. These results confirm and expand on the findings of Lina et al. (27), who selected 172 *S. aureus* isolates from those sent to the French Reference Centre for Staphylococcus Toxaemia over a 4-year period and detected the PVL genes in 93% of *S. aureus* strains associated with furunculosis and 55% of cellulitis strains but in no cases of impetigo. Other workers who used agar double immunodiffusion to detect PVL toxin in *S. aureus* isolates from patients at a single hospital in France reported that PVL-producing *S. aureus* isolates were responsible mostly for necrotizing skin infections, such as furuncles and abscesses (7, 8, 35).

TABLE 2. Characteristics of PVL-positive *S. aureus* strains

Isolate no.	Clinical detail(s)	Antimicrobial resistance[a]	Phage pattern[b]	Phage group(s)	PFGE type	ST	SCC*mec* type[c]	*agr* allele	Toxin profile
1	Pneumonia	PEN, CHL, ERY	52A, 79, 80, 75, 85, 96	I/III/V	A	1		3	*seh*
2	Cellulitis of arm	PEN, CHL, TET	42E, 53, 81	III	B	5		2	*sea, seg, sei*
3	Orbital cellulitis	PEN, MET, OXA, CHL, ERY, GEN, TET	29, 52, 52A, 79, 80, 95, 47, 53, 54, 77, 83A, 84, 85	I/III	C	8	I	1	*sec*, *seg*, *sei*, *tst*
4	Burn	PEN	29, 42E, 53, 54, 77, 83A, 85, 81, 94, 96	I/III/V	D	361		1	*sea*, *seg*, *sei*, *tst*
5	Burn	PEN, MET, OXA, TET, FUS	52, 80	I	E	80	IVc	3	*etd*
6	Skin lesions	PEN, MET, OXA, CHL, ERY, SYN, TET, FUS	79, 80	I	E	80	IVc	3	*etd*
7[d]	No information	PEN, MET, OXA	29, 52, 52A, 79, 80, 95, 6, 42E, 47, 53, 54, 75, 84, 81	I/III	E	80	IVc	3	*etd*
8[d]	No information	PEN, MET, OXA, CIP, TET, FUS	29, 52, 52A, 80, 95	I	E	80	IVc	3	*etd*
9	Epidural abscess	PEN, MET, OXA, TET, FUS	29, 52, 52A, 80	I	E	80	IVc	3	*etd*
10	Abscess	PEN, MET, OXA, TET, FUS	Nontypeable		E	80	IVc	3	*etd*
11	Family outbreak of spots	PEN, MET, OXA, TET, FUS	Nontypeable		E	80	IVc	3	*etd*
12	Boil	PEN, MET, OXA, CHL, TET, FUS	29, 52, 52A, 80	I	E	80	IVc	3	*etd*
13	Scalded skin syndrome	PEN, MET, OXA, ERY	80	I	E	80	IVc	3	*etd*
14	Shoulder abscess	PEN, MET, OXA	Nontypeable		E	80	IVc	3	*etd*
15	Skin lesion	PEN, MET, OXA, SYN, FUS	29, 80	I	F	80	IVc	3	*etd*
16	Infected tropical bites	PEN, MET, OXA, TET, FUS	Nontypeable		G	80	IVc	3	*etd*
17[d]	Bacteremia	PEN, CHL, GEN, TET	3A, 3C, 55, 71	II	H	121		4	*seb*, *seg*, *sei*
18	Perianal abscess	PEN	3A, 3C, 55, 71	II	H	121		4	*seg*, *sei*
19[d]	Skin lesions	PEN	3A, 3C, 55, 71	II	H	121		4	*seb*, *seg*, *sei*
20[d]	No information	PEN, CHL, GEN	42E, 54, 75, 83A, 81	III	I	22		1	*seg*, *sei*
21	Arm abscess	PEN, GEN, FUS	6, 42E, 47, 54, 75, 81	III	I	22		1	*seg*, *sei*
22[d]	Empyema	PEN, CHL, GEN	42E, 75, 81	III	I	22		1	*seg*, *sei*
23	Bacteremia	CHL, SYN	29, 52, 52A, 79, 80, 95, 6, 42E, 47, 53, 54, 75, 83A, 84, 85, 81, 94	I/III/V	J	243		3	*seg*, *sei*
24	Pneumonia	PEN, CHL, GEN, SYN	Nontypeable		J	360		3	*sea*, *seg*, *sei*, *tst*
25	Abdominal abscess	—	29, 6, 47, 54, 75, 85	I/III	J	30		3	*seg*, *sei*
26	Pneumonia	PEN, CHL	29, 80, 6, 47, 53, 54, 75, 77, 83A, 84	I/III	J	30		3	*seg*, *sei*
27	Cellulitis on leg	PEN, CHL, TET	29, 52, 52A, 79, 80, 95, 77, 84, 85	I/III	K	37		3	*sec*, *seg*, *sei*, *tst*
28[d]	Pneumonia	PEN, MET, OXA, CHL	29, 52, 52A, 79, 80, 95, 6, 42E, 47, 54, 75, 77, 81	I/III	L	30	IVc	3	*seg*, *sei*
29[d]	Bacteremia	PEN, CHL, GEN	29, 52, 80, 75, 84, 85, 81	I/III	M	39		3	
30	Breast abscess	—	81[e]		N	359	Nontypeable	1	

[a] The panel of antimicrobials included penicillin (PEN), oxacillin (OXA), methicillin (MET), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), linezolid (LZD), rifampicin (RIF), quinupristin-dalfopristin (SYN), tetracycline (TET), teicoplanin (TEC), vancomycin (VAN), fusidic acid (FUS), and mupirocin (MUP). —, no resistance to antimicrobials tested.

[b] Underlined phages showed strong or inhibitory reactions; the remainder were weak.

[c] SCC*mec* IVc isolates carried type 2 cassette chromosome recombinase (*ccrA*/*B*) genes (15).

[d] Isolates identified in the frequency study.

[e] Phage 81 is not assigned to a recognized phage group.

[f] Reacted with MECA P4, MECA P7, RIF5 F10, and RIF5 R13 primers only (31). No amplification product was obtained with primers for the *ccrA*/*B* genes (16).

Lina et al. (27) also found a significant association between the detection of PVL genes and community-acquired pneumonia; they did not detect the PVL genes in *S. aureus* strains from cases of hospital-acquired pneumonia. Furthermore, Gillet et al. (13) compared cases of *S. aureus* pneumonia caused by PVL-positive and PVL-negative strains and found that PVL-positive patients were younger, often had preceding influenza-like symptoms, and had poorer prognoses than PVL-negative patients. In addition, PVL-positive patients were more likely to develop hemoptysis and leucopenia. Similar cases of rapidly

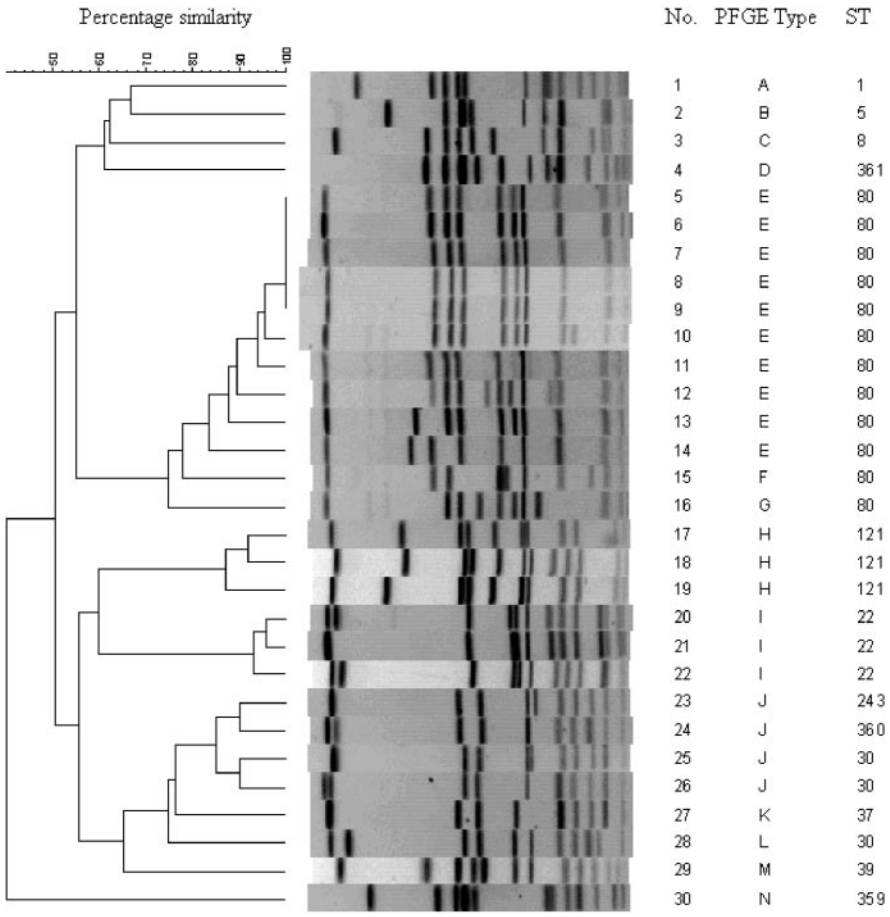

FIG. 1. PFGE patterns and phylogenetic tree of 30 *S. aureus* isolates carrying PVL genes. SmaI macrorestriction patterns were digitalized and analyzed to calculate Dice coefficients of correlation and to generate a dendrogram by unweighted-pair group method using average linkage clustering. Numbers 1 through 30 correlate with those in Table 2.

progressive, hemorrhagic, necrotizing pneumonia in young immunocompetent patients caused by PVL-positive *S. aureus* have been reported in other countries (23, 32, 42). In the present study, PVL genes were detected in 11% (4/37) of S. *aureus* strains responsible for pneumonia. Due to a lack of clinical information, it was not possible to categorize all 37 pneumonia strains into community- or hospital-acquired groups. However, we were able to establish that the four PVL-positive isolates were acquired in the community.

Isolates from patients with invasive infections, such as endocarditis and osteomyelitis, did not harbor the PVL genes, nor did isolates from patients with TSS and food poisoning, which is a result similar to the findings of Lina et al. (27).

The genetic relatedness of the PVL-positive isolates was investigated by PFGE and MLST. A good correlation between these methods was observed, although PFGE was more discriminatory. The PVL genes were present in strains with different genetic backgrounds, although a large number belonged to a single clone (ST80) that was not geographically related. ST80 strains have been recently reported to be responsible for community-acquired *S. aureus* infections in Western Europe. Vandenesch et al. (41) investigated 61 community-acquired *S. aureus* isolates from 10 different hospitals throughout France and 6 different hospitals from the Geneva area in Switzerland. They found the strains were closely related by PFGE (seven related patterns were identified) and belonged to ST80. The characteristics of their strains were very similar to those identified in the present study, namely, they were resistant to methicillin and mostly resistant to fusidic acid and tetracycline but sensitive to most non-β-lactam antibiotics. These strains also carried the SCC*mec* IV element, had an *agr*3 allele, and did not harbor the virulence genes *sea-e*, *seg-j*, *tst*, *eta*, and *etb*. Vandenesch et al. did not test their strains for *etd*, which was present in all of our ST80 strains. Also, SCC*mec* subtyping showed our isolates carried the SCC*mec* IVc element as described by Huletsky et al. (15). We cannot be sure, however, whether our strains were acquired in the community, although this seems likely.

ST80 strains showing features characteristic of community-acquired strains have also been isolated from patients in a hospital in Greece. Aires de Sousa et al. (1) detected ST80 strains in 11 patients suffering from a variety of conditions, including skin infections, bacteremia, arthritis, and cirrhosis. However, Aires de Sousa et al. were unable to confirm whether these strains were community acquired.

Most worrying was the presence of the PVL genes in strains related to the two major epidemic clones in hospitals in the United Kingdom, EMRSA-15 (ST22) and EMRSA-16 (ST36).

The three ST22s were oxacillin/methicillin sensitive; however, one of the ST30 strains that was responsible for community-acquired pneumonia was methicillin resistant and carried the SCC*mec* type IVc element. This strain is representative of the South West Pacific clone (ST30 MRSA-IV), which has been previously responsible for community-acquired infections (41). Interestingly, this clone is closely related to phage type 80/81 strains, which were responsible for serious invasive disease in healthy children and young adults in the 1950s (5, 39). Phage type 80/81 strains are ST30 and carry the genes for PVL (38).

The SCC*mec* element carried by most of the strains was identified by using the multiplex PCR described by Oliveira and de Lencastre (31). However, one isolate could not be typed by this method, and it did not appear to carry the recombinase genes *ccrA* and *ccrB*. Other workers have identified strains whose SCC*mec* elements are nontypeable (30; D. Morrison, personal communication), and recently, a novel SCC*mec*, designated type V, which carried a novel recombinase (*ccrC*) has been described (17). Interestingly, the nontypeable isolate detected in this study carried the *mecA* gene, but the expression of the gene was repressed, suggesting the isolate may be a pre-MRSA isolate.

The three phage group II strains belonged to ST121 and had an *agr*4 background. Phage group II strains and strains carrying an *agr*4 allele have been associated with exfoliatin-related diseases and typically carry *eta* and/or *etb* (18, 19, 24). Interestingly, the three phage group II, PVL-positive strains detected in this study did not harbor either of these genes, which may explain why they were not associated with a blistering disease, such as impetigo or SSS. In fact, none of the PVL-positive isolates carried *eta* or *etb*. One isolate responsible for SSS was positive for *etd*. ETD was recently described by Yamaguchi et al. (45), who showed that the ETD gene was carried on a pathogenicity island and recombinant toxin induced exfoliation of the skin in mice, similar to *eta* and *etb*. They did report, however, that the majority of *etd*-positive isolates were from sources of infections other than impetigo or SSS and hypothesized that ETD may play a broader pathogenic role in a variety of infections by destroying epithelial barriers, helping bacteria to spread or invade tissues for the exacerbation of infection. This may help explain the success of the ST80 clone, which carried the gene for ETD in combination with the genes for PVL.

In conclusion, our study has revealed that the PVL genes are carried by a relatively low number of *S. aureus* isolates referred from clinical laboratories throughout England and Wales. These isolates were associated mostly with necrotic infections of the skin and soft tissue but were also detected in patients with community-acquired pneumonia. The PVL-positive isolates were from diverse genetic backgrounds, but a prevalent clone that has been responsible for community-acquired infections in other European countries was identified. Currently, PVL-positive community strains are not spreading in hospitals. However, the discovery of the PVL genes in isolates closely related to the epidemic clones currently circulating in United Kingdom hospitals is a cause for concern. Close surveillance of these strains is essential to monitor their spread, antimicrobial resistance profiles, and association with disease.

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